Supporting Information

Ultrafast fluorescence spectroscopy reveals a dominant weakly-emissive population of fibril bound Thioflavin-T

METHODS

Thioflavin T (ThT) was obtained from Sigma-Aldrich as the chloride salt of the dye and was re-crystallized twice from methanol. The purity of the re-crystallized ThT was checked through NMR spectra. Bovine insulin and sodium chloride was also obtained from the Sigma-Aldrich and was used as received.

Preparation of insulin fibrils

It is well-known that Bovine insulin forms fibril at low pH and elevated temperature.[1] Solutions of bovine insulin (2 mg/mL) in 20% acetic acid were freshly prepared and incubated at 70 °C for 24 h in a glass vial under stirring. A stock solution of ThT was freshly prepared in nanopure water and was added to fibril solution to make a final concentration of ThT of 8 μM. The solution was incubated at room temperature for 1 h before final measurements.

Ground-state absorption and steady-state emission measurements

Ground-state absorption spectra were recorded using a Shimadzu spectrophotometer (Model V-650). Steady-state fluorescence measurements were carried out in a Hitachi spectrofluorimeter, model F-4500.

Time-resolved measurements

The time-resolved fluorescence measurements, in the nanosecond time domain, were carried out using a diode laser based time-correlated single-photon counting (TCSPC) spectrometer from IBH, U.K. A 408 nm diode laser (1 MHz repetition rate) was used for sample excitation. A microchannel plate (MCP) detector was used for the detection of the emitted photons through a monochromator. The IRF of the TCSPC instrument was measured by collecting the scattered light from a TiO_2 suspension in water and was found be ~100 ps. The lifetime measurements were done at magic angle condition.

Time-resolved fluorescence measurements, in the sub-picosecond time domain, were carried out using a femtosecond fluorescence upconversion instrument (FOG 100, CDP Inc. Russia) which has been described earlier.[2] Briefly, samples were excited using a second harmonic laser pulse (410 nm, 50 fs, 88 MHz) after frequency doubling of the fundamental output (820 nm) of a Ti-Sapphire oscillator. The remaining fundamental laser beam was used as gate beam. The fluorescence signal collected from the sample was upconverted by overlapping with the gate beam into a BBO crystal after passing through a delay rail. The upconverted signal is allowed to pass through a bandpass filter to cut off excitation and gate beams and was dispersed in a double monochromator. The instrument response function (IRF) was independently measured through the cross correlation of the fundamental and the excitation laser pulse. The IRF was found to have a Gaussian intensity profile with FWHM of 220 fs. All the decay traces were collected at magic angle polarization with respect to the horizontal excitation pulse to remove any contributions from rotational reorientation on the decay traces. The reproducibility of the measurement was checked by measuring each decay trace 2-3 times. Sample was taken in a rotating cell of path lengh 0.4 mm to avoid the photo-degradation of the sample.

The decay traces are fitted with a multi-exponential function of the following form,

$$I(t) = I(0)\sum_{i=1}^{n} \alpha_i \exp(-t/\tau_i) \tag{1}$$

The mean fluorescence lifetime is calculated according to the equation[3]

$$<\tau> = \sum_{i=1}^{n} \alpha_i \exp(-t/\tau_i) \quad \text{where} \quad <A_i> = \alpha_i\tau_i / \sum \alpha_i\tau_i \tag{2}$$

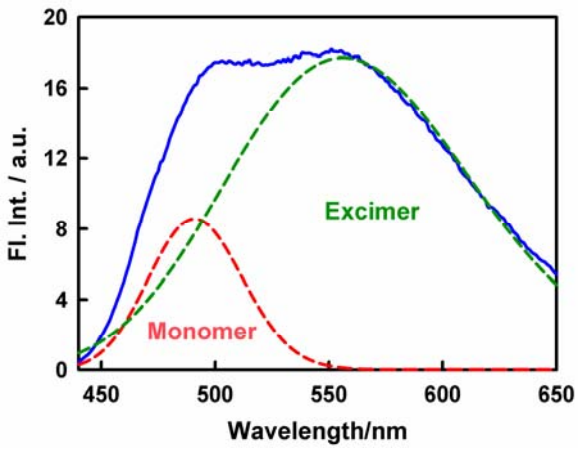

Figure S1. Steady-state fluorescence spectrum of ThT in γ-Cyclodextrin. The solid blue line is the experimental spectrum. A two Gaussian fitting is employed to deconvolute the spectra of momomer and excimer from of ThT. The broken red line corresponds to the emission from the monomeric ThT and the broken green line corresponds to the emission from ThT excimer.

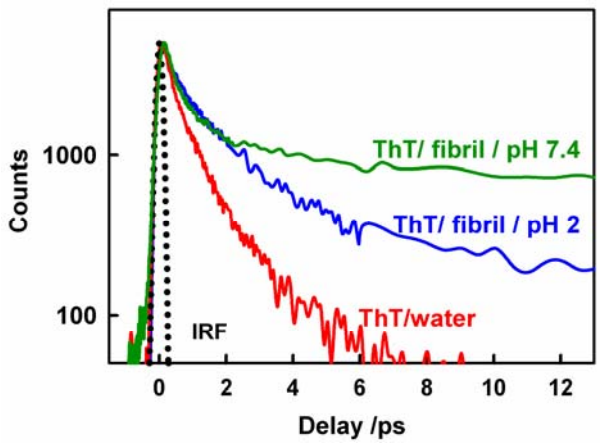

Figure S2. Transient decay trace measured by Fluorescence up-conversion technique (λ_{em}= 490 nm) for ThT in water (solid red line), insulin amyloid fibril at pH 2 (solid blue line), and insulin amyloid fibril at pH 7.4 (solid green line). IRF stands for instrument response function.

References

1. M. Manno, D. Giacomazza, J. Newman, V. Martorana and P. L. S. Biagio, *Langmuir*, 2010, **26**, 1424-1426.
2. P. K. Singh, S. Nath, A. C. Bhasikuttan, M. Kumbhakar, J. Mohanty, S. K. Sarkar, T. Mukherjee and H. Pal, *J. Chem. Phys.*, 2008, **129**, 114504.
3. J. R. Lakowicz, *Principle of fluorescence spectroscopy*, Plenum Press, New York, 2006.